USE OF STRAIN OF BACILLUS AND BYPRODUCTS THEREOF FOR INHIBITING FORMATION OF BLOOD VESSELS

FIELD OF THE INVENTION

The present invention is related to the field of probiotics useful in the prevention of disorders.

BRIEF DESCRIPTION OF THE PRIOR ART

Cancer research aims to discover means by which the aggressive growth of solid tumours and their metastases can be abolished in a specific way without causing treatment resistance, or provoke excessive toxicity in treated patients. The challenge is high, since the transformation of normal cells into tumour cells is associated with the acquisition of resistance to most cytotoxic agents presently used in therapy. Several studies done in the last few years have demonstrated that tumour cells do not represent the only factor responsible for tumour growth. Blood vessels present within these tumours play also a crucial role. It has been clearly established that blood vessels, formed by the angiogenesis process (Figures 15 and 16), are essential to aggressive growth of tumours and their metastases. This angiogenesis is due to the capacity of tumour cells to secrete a certain number of angiogenic factors, like vascular endothelium growth factor (VEGF) and fibroblastic growth factor (FGF), linking with high affinity the surface of endothelial cells. The stimulation of endothelial cells by these factors, results not only in an increase of secretion of enzymes degrading the extra cellular matrix components, but also in the stimulation of the migration and the proliferation of these cells. The thus stimulated cells invade the matrix surrounding the tumours, forming a capillary network which will ensure the growth of tumour cells, by giving them nutrients and oxygen necessary for their development. The inhibition of blood contribution to the tumours constitutes thus a target of choice for the development of new therapeutic anticancerous approaches targeting specifically angiogenesis to limit or eliminate tumour progression.

It is estimated that life habits and eating habits are responsible for more that one third of new diagnosed cancers. Consequently, prevention (Nutra-prevention) presently creates a big interest and it is estimated that in the following few years, it will bring reduction in

4. Daoy (brain medulloblastoma)
5. U-87 (brain glioblastoma-astrocytoma)
6. Jurkat (leukemia lymphocytes)

These studies will permit to better characterize and identify new molecular targets, modulated by BIO-K-Plus, endothelial cells and cancerous cells modulated by BIO-K-Plus.

1.1 Preparation of study material

In the present study, the inventors have characterized the supernatants action of irradiated lactic acid bacteria at 3 kGy (S3), 6 kGy (S6) and 9 kGy (S9). These supernatants have been obtained after two centrifugations (one at 6 000g for 15 min at 4°C and the other at 10 000g for 20 min at 4°C). They have then been filtered on two filters (on filter of 0.05 µm followed by a filter of 0.22 µm) to obtain bacteria free sterile supernatants and in order to be able to treat divers cells lines. Supernatants have been kept at -80°C until use.

For those studies, the inventors have used a concentration of supernatants equivalent to 10^8 bacteria, since it is at this concentration that the inhibitor effect is maximal.

1.2 *In vitro* characterization of antiangiogenic properties of BIO-K-Plus on HUVECs.

The inventors have verified if bacterial supernatants have an effect on endothelial cells. The WST-1 technique, which measures mitochondrial activity of cells, has permitted the study of the cell proliferation of HUVECs. The supernatants did not seem to have an inhibitory effect on the cells proliferation (Figures 8 and 9; n=2). The inventors have then evaluated the migratory potential of cells in presence of bacterial supernatants and the results have been positive. The inventors have verified if supernatants inhibit the stimulation of HUVECs migration on gelatine induce by VEGF, the mitogen the most often associated with angiogenesis phenomena. The supernatants inhibit completely the migration by VEGF but also the basal level of migration at approximately 50% (Figure 10B). The inhibitor effect of supernatants does not seem to be specific to VEGF. The assays of tube formation on Matrigel (in laminin rich matrix, reconstituting the basal

membrane and which permits the endothelial cells differentiation in similar structures to capillary blood vessels) demonstrate that bacterial supernatants inhibit in a significant way the tube formation compared to a control in HUVECs (Figures 11A and 11B; n=2). These results indicate that bacterial supernatants contain molecules which have an antiangiogenic potential.

1.3 Conclusion

The inventors have demonstrated that the supernatants coming from lactic acid bacteria containing *Lactobacillus acidophilus* and *Lactobacillus casei* has an antiangiogenic activity.